

## Simultaneous for the estimation of irbesartan (IRBES) and hydrochlorothiazide (HCTZ) in pharmaceutical formulations by UFLC method

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**Abstract:** A simple, rapid, sensitive, specific, precise and accurate Ultra-Fast liquid chromatography (UFLC) method was developed for simultaneous determination of Irbesartan and Hydrochlorothiazide in Pharmaceutical formulations. The chromatographic separation was conducted on Shimadzu (Prominence LC 20 UFLC XR) connected with photodiode array (PDA) detector; using ACE column, C18/CN (100 x 4.6 mm, 5  $\mu$ m) as stationary phase. Isocratic mobile phase consisted of Methanol and 0.1% Phosphoric acid 85% in the ratio of (55:45 v/v); at flow rate of 1.5 ml min<sup>-1</sup> was used. An injection volume of 20  $\mu$ L was used for both Irbesartan and Hydrochlorothiazide. The detection wavelength ( $\lambda_{max}$ ) was 225 nm using a diode array detector. Linearity of the method was established over the concentration ranges of 60 – 600  $\mu$ g ml<sup>-1</sup> for Irbesartan, with a retention time of 2.10 minutes and 5 – 50  $\mu$ g ml<sup>-1</sup> for Hydrochlorothiazide, with a retention time of 0.88 minutes. Correlation coefficients were greater than 0.999. The relative standard deviation (RSD) was found to be < 2. The method can be used successfully for simultaneous determination of Irbesartan and Hydrochlorothiazide in pharmaceutical formulations.

**Keywords:** UFLC, Irbesartan, Hydrochlorothiazide, Mixed column, Method validation.

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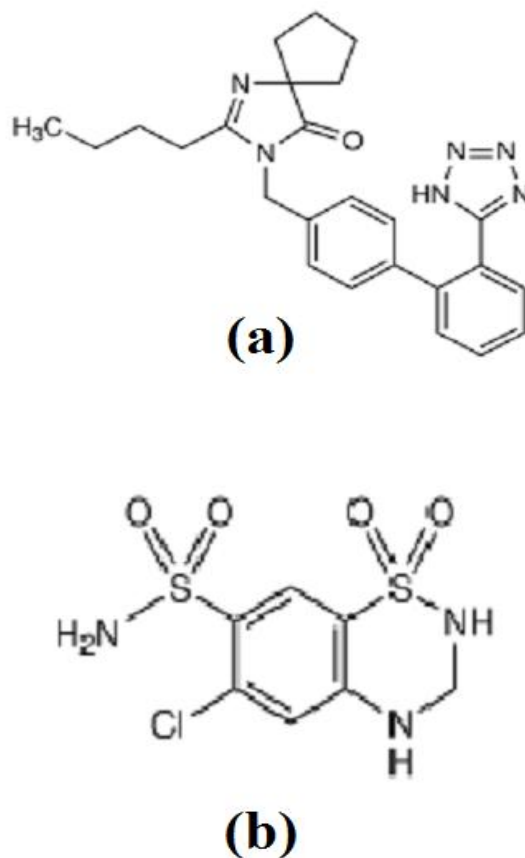
### I. Introduction

Hypertension is widespread disease with a major risk factor for stroke and to some extent ischemic heart disease. Antihypertensives have differing pharmacological actions. Different categories are used for treatment of hypertension i.e. diuretics and angiotensin II receptor antagonist.

Irbesartan (IRBES) is an angiotensin II receptor antagonist; indicated for hypertension treatment; used also for delaying progression of diabetic nephropathy and reduction of renal disease progression in patients with type 2 diabetes, hypertension and microalbuminuria (> 30 mg/24 h) or proteinuria (> 900 mg/24 h) [1, 2]. Irbesartan is also available in a combination formulation with a low-dose thiazide diuretic, as hydrochlorothiazide, to achieve an additive antihypertensive effect. Irbesartan is rapidly absorbed from the gastrointestinal tract with an oral bioavailability of 60 to 80%. Peak plasma concentrations of irbesartan occur 1.5 to 2 hours after an oral dose. Irbesartan is about 96% bound to plasma proteins. It undergoes some metabolism in the liver [3].

Hydrochlorothiazide (HCTZ) is a thiazide class of diuretic, used to treat hypertension and swelling due to fluid build-up. Other uses include congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis and to decrease the risk of kidney stones in those with high calcium level in the urine. It is often recommended as a first line treatment for hypertension treatment [4-6]. HCTZ is taken orally and may be combined with other blood pressure medications as a single pill to increase the effectiveness. Being a thiazide medication class; it acts by decreasing the kidneys ability to retain water. This initially reduces blood volume, decreasing blood return to the heart and thus cardiac output. Long term, however, it is believed to lower peripheral vascular resistance [7]. It is also used for the prevention of kidney stones in those who have high levels of calcium in their urine. It is also sometimes used for treatment of hypoparathyroidism, hypercalciuria, Dent's disease, and Ménière's disease [8]. Thiazides are also used in the treatment of osteoporosis by decreasing mineral bone loss by promoting calcium retention in the kidney and by directly stimulating osteoblast differentiation and bone mineral formation [9]. It reduces blood volume by acting on the kidneys to reduce sodium reabsorption in the distal convoluted tubule. Thiazides increase the reabsorption of calcium in this segment in a manner unrelated to sodium transport [10]. Additionally, by other mechanisms, HCTZ is believed to lower peripheral vascular resistance [11].

Different literatures were published concerning the estimation of Irbesartan and Hydrochlorothiazide<sup>-</sup> combined [12-15].



**Fig. 1** Chemical structures of Irbesartan (a) and Hydrochlorothiazide (b).

The present work was to develop a simple, rapid, sensitive, and cost-effective UFLC method for routine analysis. The proposed method was validated according to ICH guidelines [16].

## II. Materials and Methods

### Chemicals and reagents

Irbesartan and Hydrochlorothiazide were obtained from Hetero Drugs, Hyderabad, (India). Methanol HPLC grade was purchased from Fisher Chemical, (UK). Ortho-Phosphoric acid 85% was HPLC grade from Fluka chemicals, (Germany). Acetonitrile HPLC grade was purchased from J. T. Baker, (UK). Water for chromatography was purchased from Merck, (Germany). Mobile phase was filtered using 0.45  $\mu$ m nylon membrane filter, ChromTech, (UK).

### Equipment and chromatographic conditions

The analysis of drugs was carried out on a Shimadzu LC-20 UFLC XR, prominence (Kyoto, Japan) equipped with an auto sampler (SIL-20AC XR, Shimadzu, Japan) and PDA detector (SPD- M20A, Japan) was used for the analysis. Peak areas were integrated using a Shimadzu LC solution (Version 5.41.240) software program. The data was recorded using LC-solution software. A NSXX sonics ultrasonic bath (NS-A-12-7H, Germany) was used for degassing of the mobile phase.

The HPLC separation and quantitation were achieved on a 100x4.6 mm ACE C18/CN (5  $\mu$ m particle size) column (Scotland). The mobile phase was prepared by methanol : 0.1% phosphoric acid 85% in ratio of (55:45) (v/v) which was run isocratic. The mobile phase was delivered to the system at a flow rate of 1.5 ml/min. All determinations were performed at ambient temperature. The injected volume was 20  $\mu$ l. The detector was set at 225 nm. The run time was set for 3.0 min. The optimized chromatographic condition is shown in Table 1.

**Table 1** Optimized chromatographic conditions

Parameters	Conditions
Stationary phase	ACE, C18/CN, 100 x 4.6 mm, 5 µm
Mobile phase	Methanol and 0.1% H <sub>3</sub> PO <sub>4</sub> (55:45 v/v)
Flow rate (mL min <sup>-1</sup> )	1.5
Run time (min)	3.0
Column temperature (°C)	Ambient (25 °C)
Injection volume (µL)	20
Detection wavelength (nm)	225nm
Retention time of IRBES (min)	2.10
Retention time of HCTZ (min)	0.88

**Preparation of standard stock and standard solution**

Standard solutions of either were prepared by dissolving 300 mg irbesartan standard and 25 mg hydrochlorothiazide standard in 100 ml acetonitrile and water at the ratio of (1:1% v/v) and shaken on vortex for 2 min. Then it was sonicated for 10 minutes. Concentration range of irbesartan and hydrochlorothiazide from 60.0 – 600.0 µg/ml and 5.0 – 50.0 µg/ml; respectively was used. The solution was filtered through a 0.45 µm nylon filter before analysis.

**Linearity**

Linear calibration plots of the proposed method were obtained over concentration ranges of 60-600 µg.ml<sup>-1</sup> (60, 150, 240, 300, 480 and 600 µg ml<sup>-1</sup>) for irbesartan and 5-50 µg ml<sup>-1</sup> hydrochlorothiazide (5, 12.5, 20, 25, 40 and 50 µg ml<sup>-1</sup>). Each solution was prepared in triplicate.

**Accuracy**

Accuracy was evaluated by spiking standard with sample solution. The measurements were made at the concentration of standard mix; which was found to be the target concentration and at suitable intervals around this point. The test samples was spiked with known quantities of standard irbesartan and hydrochlorothiazide using three determinations over three concentrations level covering the specified range. Relative recoveries of standard irbesartan and hydrochlorothiazide; used in the standards were evaluated by comparing their peak area with those obtained from the calibration curve equation.

**Specificity**

It provides an indication of the selectivity and specificity of the procedure. The method is to be selective, if the main peak is well resolved from any other peak by resolution of minimum 2. This was done by injecting placebo and comparing it with that of standard and placebo; spiked with standard and sample. Then the peak purity was ascertained using of PDA.

**System suitability**

System suitability was performed by injecting six replicates of standard solution at 100% of the test condition at a 100% level to verify the precision of the chromatographic system. The purposed UFLC method permits the concurrent determination of irbesartan and hydrochlorothiazide in sample drug; through having different retention times. System suitability data are given in Table 2.

**Table 2** System suitability parameters for Irbesartan and Hydrochlorothiazide

S. No.	Parameters	HCTZ	IRBES
1	Tailing factor	1.06	1.12
2	Retention time	0.88	2.10
3	Theoretical plates	230	685

### Ruggedness

It's defined the degree of reproducibility of test results obtained by the analysis of the same samples under variety of conditions such as different analysts, different columns, different days etc.

- **Day to day:** Five replicates of a single sample of powder material (100%) were used for each determination. On the first day; five replicates was analyzed. Then, on the second day, another five replicates of freshly prepared test from the same sample were analyzed by same analyst.
- **Analyst to analyst:** It determines ruggedness between different analysts. Five replicates of a single sample were analyzed. Then, a second person analyzed five replicates from the same sample, prepared by him.
- **Column to column:** The same analytical method was performed on columns of the same packing material and length but of different batch number.

### Robustness

Robustness is determined by observing how a method stands up to slight variations in normal operating parameters. For instance, for HPLC, this could change if slight variation in sonication time or in aliquot stability.

### Limit of detection (LOD) and limit of quantitation (LOQ)

Detection and quantitation limits were determined by the signal-to-noise (S/N) approach. In order to examine the limit of quantitation and limit of detection, solutions of different concentrations were prepared by spiking known amounts of irbesartan and hydrochlorothiazide. Each solution was prepared according to the defined protocol and analyzed repeatedly to determine the S/N ratio. The average S/N ratio from all the analyses at each concentration level was used to calculate the limit of quantitation and limit of detection. The concentration level that gives an S/N ratio of 10:1 at which analytes can be readily quantified with accuracy and precision was reported as the limit of quantitation. The concentration level that gives an S/N ratio of 3:1 at which analytes can be readily detected was reported as the limit of detection.

## III. Results and Discussion

The proposed UFLC method required fewer reagents and materials, and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatogram of irbesartan and hydrochlorothiazide was shown in Fig. 2. There was clear resolution between irbesartan and hydrochlorothiazide with retention time of 0.88 and 2.10 minutes; respectively. The developed chromatographic method was validated using ICH guidelines [16]. Validation parameters include linearity, accuracy, precision, robustness, specificity, limit of detection and quantitation.

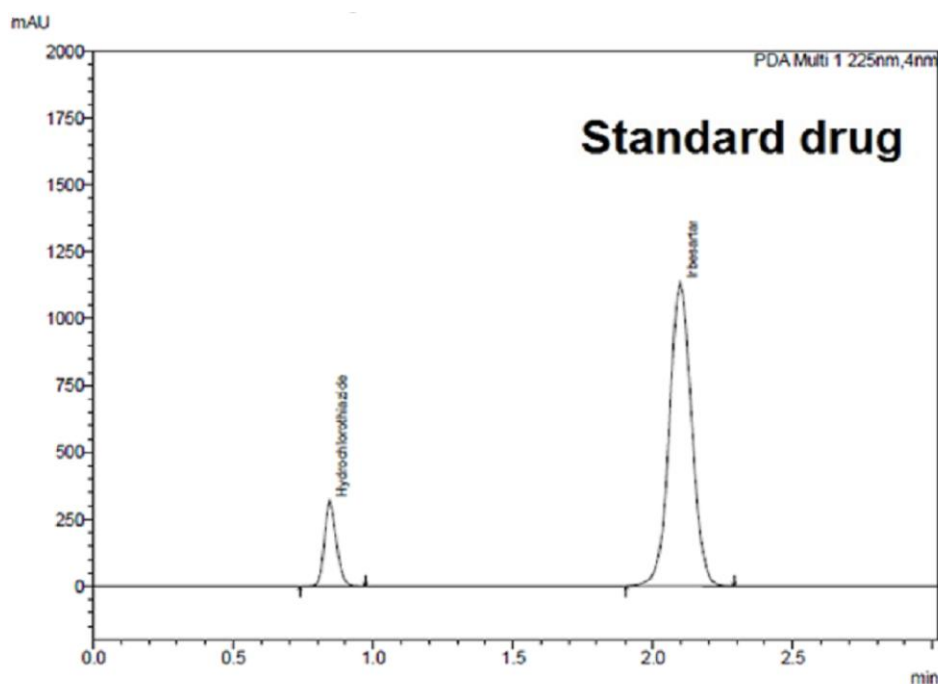
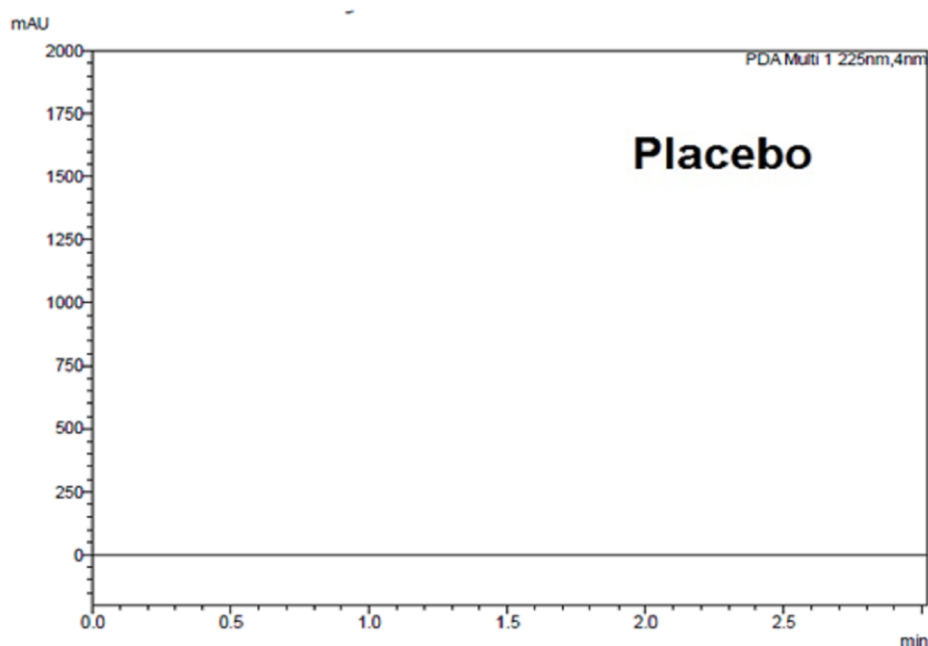
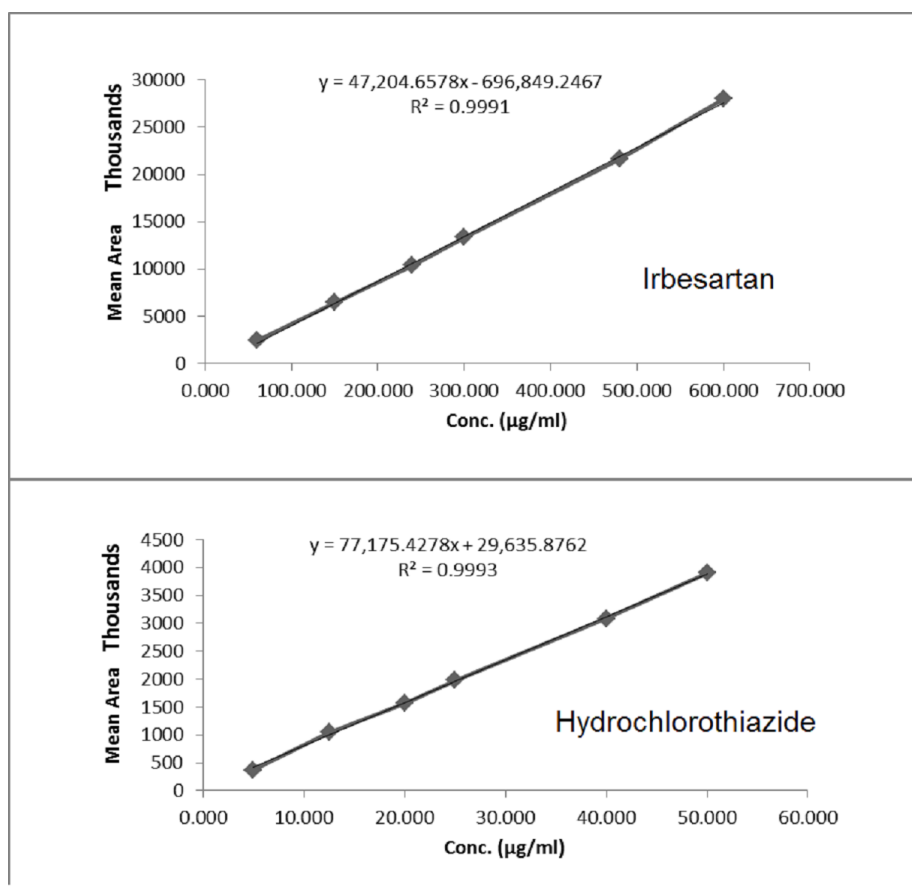


Fig 2. UFLC chromatogram for Irbesartan and Hydrochlorothiazide (standard drug).



**Fig 3.** UFLC chromatogram for Irbesartan and Hydrochlorothiazide (placebo).

Linear calibration plots for the proposed method were obtained in concentration ranges of 60-600  $\mu\text{g mL}^{-1}$  (60, 150, 240, 300, 480 and 600  $\mu\text{g mL}^{-1}$ ) for irbesartan as shown in Fig. 4 and data are shown in Table 3 and 5-50  $\mu\text{g mL}^{-1}$  hydrochlorothiazide (5, 12.5, 20, 25, 40 and 50  $\mu\text{g mL}^{-1}$ ) as shown in Fig. 4 and data are shown in Table 3.



**Fig. 4** Calibration curve of Irbesartan and Hydrochlorothiazide.

**Table 3** Statistical data of calibration curves of Irbesartan and Hydrochlorothiazide

S. No.	% Working Concentration		Concentration ( $\mu\text{g mL}^{-1}$ )		Average peak area	
	IRBES	HCTZ	IRBES	HCTZ	IRBES	HCTZ
1	25	25	60	5	76705	90807
2	62.5	62.5	150	12.5	390321	435428
3	100	100	240	20	785694	908160
4	125	125	300	25	1585150	1750145
5	200	200	480	40	1944125	2157840
6	250	250	600	50	2290374	2567566
<b>Regression co-efficient (IRBES) = 0.9991</b>						
<b>Regression co-efficient (HCTZ) = 0.9993</b>						

Each of the concentrations was injected in triplicate to get reproducible response. Calibration curves were constructed by plotting peak area versus concentration. Each reading was average of three determinations. They were represented by the linear regression equation.

$$Y_{\text{Irbesartan}} = 47204.6578x - 696849.2467, r^2 = 0.9991$$

$$Y_{\text{Hydrochlorothiazide}} = 77175.4278x + 29635.8762, r^2 = 0.9993$$

Slopes and intercepts were obtained by using regression equation ( $Y = mx + c$ ) and least square treatment of the results used to confirm linearity of the method developed.

The limit of detection (LOD) and quantitation (LOQ) were determined by making serial dilutions. LOD was found to be  $10.0 \mu\text{g.mL}^{-1}$  and  $0.833 \mu\text{g.mL}^{-1}$  for irbesartan and hydrochlorothiazide; respectively (signal to noise ratio of 3:1). LOQ was found to be  $30 \mu\text{g.mL}^{-1}$  and  $2.50 \mu\text{g.mL}^{-1}$  for irbesartan and hydrochlorothiazide; respectively (signal to noise ratio of 10:1).

Accuracy was calculated by addition of standard drugs to preanalyzed sample at 3 different concentration levels and computing percentage recoveries. Standard limit of % recovery study is 98 - 102 % as per ICH guideline. From the studies it was concluded that % recovery study of irbesartan and hydrochlorothiazide complies with standard limit of ICH guideline. Results of accuracy were proven by the Table 4.

**Table 4** Accuracy and recovery results for determinations of Irbesartan and Hydrochlorothiazide

IRBES				HCTZ			
Working conc. ( $\mu\text{g/ml}$ )	Peak area	Found conc. ( $\mu\text{g/ml}$ )	% Recovery	Working conc. ( $\mu\text{g/ml}$ )	Peak area	Found conc. ( $\mu\text{g/ml}$ )	% Recovery
150	6442652	151.11	100.74	12.5	1002451	12.61	100.84
	6453264	151.47	100.98		1004712	12.63	101.08
	6412575	150.61	100.41		1001347	12.59	100.73
240	10562412	241.29	100.54	20	1592874	20.26	101.28
	10754845	242.60	101.08		1547845	19.67	98.36
	10762542	242.76	101.15		1569845	19.96	99.79
480	21829574	476.81	99.34	40	3088754	39.64	99.10
	21881102	478.30	99.65		3098547	39.77	99.41
	21721540	474.92	98.94		3056421	39.22	98.05

Specificity of a method is its suitability for the analysis of a compound in the presence of potential impurities. Placebo, standards, and sample test solutions were all injected at the same wavelength of 225 nm to demonstrate the specificity of the optimized method. A comparison of the retention times of irbesartan and hydrochlorothiazide in sample solutions and in the standard solutions were exactly the same. Fig. 2 & 3 showed that there were no interferences at the retention times for irbesartan and hydrochlorothiazide due to the placebo. Therefore, the proposed method is suitable for the quantification of the active ingredients in tablet formulation.

#### IV. Conclusion

A simple, sensitive, fast, isocratic and accurate UFLC method is described for simultaneous determination of irbesartan and hydrochlorothiazide in pharmaceutical formulations. As a result, the proposed UFLC method could be adopted for the quantitative quality control and routine analysis of tablet dosage form.

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