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Research Article

SIMULTANEOUES ESTIMATIION OF IRBESARTAN AND HYDROCHLORTHIAZIDE BY RP –HPLC METHOD

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ABSTRACT

A reliable Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was used in this study to estimate both Irbesartan and Hydrochlorothiazide simultaneously. A C18 column was used for efficient separation, with a buffer and organic solvent in an appropriate ratio as the mobile phase. Validation followed ICH Q2(R1) guidelines to ensure accuracy, precision, specificity, linearity, robustness, and system suitability. Across both Irbesartan and Hydrochlorothiazide's concentration ranges, the developed method showed excellent linearity. Recoveries confirmed the method's accuracy, and precision studies demonstrated its reproducibility. Hydrochlorothiazide and Irbesartan in combined dosage forms can be routinely analyzed with this validated RP-HPLC method.

Keywords: - Irbesartan, Hydrochlorothiazide, RP-HPLC, Validation, Pharmaceutical Dosage Form.

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INTRODUCTION

Irbesartan and hydrochlorothiazide are commonly used antihypertensive agents in the management of hypertension and related cardiovascular conditions. Irbesartan, an angiotensin II receptor blocker (ARB), works by inhibiting the action of angiotensin II, which helps relax blood vessels and reduce blood pressure [1]. Hydrochlorothiazide, a thiazide diuretic, helps in lowering blood pressure by promoting diuresis and reducing fluid retention [2]. The combination of these two drugs is frequently prescribed to enhance therapeutic efficacy and manage hypertension more effectively [3].

Accurate simultaneous estimation of irbesartan and hydrochlorothiazide is crucial due to their combined therapeutic use in fixed-dose formulations. These formulations are designed to improve patient compliance and ensure consistent therapeutic outcomes [4]. The simultaneous quantification of these drugs is essential for quality control during manufacturing, ensuring that each dose contains the correct amounts of both active ingredients. Additionally, such analytical methods help in monitoring the stability of the drug formulations and ensuring their efficacy over time [5].

Developing an analytical method for the simultaneous estimation of irbesartan and hydrochlorothiazide presents several challenges. These include ensuring adequate separation of the compounds from each other and from potential impurities, achieving high sensitivity and selectivity, and developing a method that is robust under various conditions [6]. The presence of excipients and other formulation components can complicate the analysis, necessitating a method that effectively separates the drugs from these substances [7].

Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) is a widely used technique for the simultaneous estimation of multiple compounds due to its high resolution, sensitivity, and versatility [8]. RP-HPLC is particularly well-suited for separating non-volatile and polar compounds, making it ideal for analyzing irbesartan and hydrochlorothiazide [9].

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The choice of mobile phase, column type, and detection wavelength are critical factors in optimizing RP-HPLC methods for accurate and reliable analysis [10].

Several studies have explored RP-HPLC methods for the simultaneous estimation of irbesartan and hydrochlorothiazide. For instance, Sharma et al. (2020) developed an RP-HPLC method that successfully separated these drugs in tablet formulations, validating the method according to ICH guidelines for accuracy, precision, and robustness [11]. Another study by Gupta and Singh (2021) focused on optimizing the chromatographic conditions to enhance resolution and reduce analysis time, demonstrating the method's applicability for routine quality control [12].

Validation of the RP-HPLC method is a critical step to ensure that it meets the required standards for accuracy, precision, specificity, linearity, and robustness. According to ICH guidelines, method validation helps confirm that the analytical method provides reliable and reproducible results, making it suitable for its intended purpose [13]. Validation is essential for compliance with regulatory requirements and for ensuring the quality and safety of pharmaceutical products [14].

The primary objective of this study is to develop and validate an RP-HPLC method for the simultaneous estimation of irbesartan and hydrochlorothiazide in tablet dosage forms. The study aims to optimize chromatographic conditions to achieve effective separation of the two drugs, validate the method for various performance parameters, and ensure its applicability for routine analysis in pharmaceutical quality control [15].

METHODOLOGY

Materials used

Instruments used in research work

HPLC Used: LC 2010C, Shimadzu (Shimadzu Corporation, Japan)
Analytical Balance: AX 205, METTLER TOLEDO
pH Meter : Thermo Orion, model 420
Sonicator : Oscar Ultra Sonics, OU- 72 (SPL)
UV Used: UV 1800, Shimadzu (Shimadzu Corporation, Japan)

Experimental work

Chromatographic condition column: ACE C18 AR 4.6 X 250 mm, 5µm.
Detector: 280 nm
Injection Volume: 10 µl
Flow Rate: 1.0 mL min-1
Temperature: 30º C
Run Time: 12 minutes
Mobile Phase: Sodium perchlorate Buffer (at pH 3.0): Acetonitrile (60:40, v/v)
Diluent: Methanol

Buffer preparation:

Accurately weighed 4.3 g sodium perchlorate was dissolved in to 1000 mL milli-q water and 1ml triethylamine was added to this buffer solution, than pH was adjusted to 3.0 with orthophosphoric acid.

Preparation of standard solution:

The standard stock solution Irbesartan (200 µg/ml) and Hydrochlorthiazide (250 µg/ml) were prepared by weighing Irbesartan 40 mg and Hydrochlorthiazide 50 mg in 200 ml volumetric flask respectively. Add 80 ml diluent to it and sonicate well for 10 minutes and making volume up to mark with diluent. Then 10 ml of standard stock solution was diluted to 100 ml with diluent to make final standard concentration of Irbesartan (20 µg/ml) and Hydrochlorthiazide (25 µg/ml), respectively.

Preparation of test solution:

Accurately 20 marketed tablets were weighed and average weight of tablet was calculated. Then tablets were finely crushed, powdered and sample powder about 1400 mg (Tablet powder Equivalent to 40 mg Irbesartan and 50 mg Hydrochlorthiazide or four tablets powder) was transferred into 200 ml volumetric flask. Then add about 100.0 ml diluent was added and sonicated for 40 minutes with intermittent shaking. Then volume was made up to mark with diluent. Then 10 ml of standard stock solution was diluted to 100 ml with diluent to make final standard concentration of Irbesartan (20 µg/ml) and Hydrochlorthiazide (25 µg/ml), respectively. The test solution was filtered through 0.45µ (PVDF Millipore Filter) and analyzed by using HPLC. RP-HPLC chromatogram of Irbesartan and Hydrochlorthiazide is shown in Figure 1.

Method validation

Validation was carried out with respect to various parameters, as required under ICH guideline Q2 (R1). The developed method validated with respect to parameters such as system suitability, solution stability, specificity, linearity, repeatability, accuracy, intermediate precision, robustness, limit of detection and limit of quantitation.

System suitability and system precision

System suitability test were performed to check repeatability of system for particular analysis performed. The results for system suitability parameters were found satisfactory. The results of system suitability and system precision are presented in Table 2.

Solution stability

Standard and sample solutions were kept at room temperature (25°C) for 24 hours and solution stability data after 0 hours, 8 hours and 24 hours were calculated. The change in % RSD was calculated. Standard solution and sample solution of concentration of Irbesartan (20 µg/ml) and Hydrochlorthiazide (25 µg/ml) were taken to test solution stability. It was found that change in % RSD for standard and sample solution was not more than 2%. The results of solution stability are summarized in Table 3 and Table 4. The standard and sample solutions were found stable up to 24 hours at room temperature.

Specificity

Specificity of analytical method is ability to measure analyte accurately and specifically in presence of component that may be expected to be present in the sample matrix. Chromatograms of standard, sample, blank and placebo were overlaid for specificity check in sample. Peak purity of standard and sample were obtained using photodiode array detector. The results of peak purity showed peak purity index greater than 0.99. The results of specificity of Irbesartan and Hydrochlorthiazide are summarized in Table 5.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well-defined mathematical transformation) proportional to the analyte concentration in samples within a given range. Linearity usually expressed in terms of the variance around the slope of regression line calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte. To achieve linearity range, stock solution containing Irbesartan (200 µg/ml) and Hydrochlorthiazide (250 µg/ml) were prepared. Irbesartan and Hydrochlorthiazide stock solutions were diluted to yield solutions in the concentration range of 5-30 µg mL-1 and 6.25-37.5 µg mL-1, respectively. The solutions were analyzed by using HPLC. Calibration curve for both the drugs are shown in the results of linearity are presented in Table 6.

Repeatability:

The method repeatability was done by preparing six different sample preparations by one analyst. The results are presented in Table 6.7. The results obtained were within 2% RSD.

Intermediate precision

Intermediate precision test was determined between different analyst, instrument and column. The value of percentage RSD was below 2.0%, showed Intermediate precision of developed analytical method. The results are presented in Table 7.

Accuracy

The difference between theoretical added sample amount to the placebo and practically achieved sample amount from placebo (after HPLC analysis) is called accuracy of analytical method. Accuracy was determined at three different level 50%, 100% and 150% of the target concentration in triplicate. The results are presented in Table 6.8 and Table 6.9.

Robustness

Robustness of the method was carried out by deliberately made small changes in the flow rate, pH, organic phase ratio and column oven temperature. Results are presented in Table 10.

LOD (Limit of Detection and LOQ (Limit of Quantitation)

In order to estimate the limit of detection (LOD) and limit of quantitation (LOQ) values, the blank sample was injected six times and the peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level while ten times the noise value gave the LOQ [16, 17]. The results of LOD and LOQ are mentioned in Table 11.

Table 1: Chemicals used in research work.

Name of chemical	Grade	Company
Water	HPLC	In house
Triethylamine	HPLC	Merck
Acetonitrile	HPLC	Merck
Sodium perchlorate	Analytical reagent	Spectrochem
Ammonium acetate	Analytical reagent	Merck
Potassium dihydrogen phosphate	Analytical reagent	Merck
Sodium Hydroxide	Analytical reagent	Merck
Orthophosphoric acid	HPLC	Merck
Chloroform	HPLC	Merck
Toluene	HPLC	Merck
Glacial acetic acid	HPLC	Merck

Methanol	HPLC	Merck
Sodium dihydrogen Phosphate	Analytical reagent	Merck
Ammonia	Analytical reagent	Merck
Ethyl acetate	HPLC	Merck

Table 2: System suitability parameters of RP-HPLC analysis for Irbesartan and Hydrochlorthiazide.

Compound	Retention Time ± SD (min) (N=3)	Theoretical plates ± SD (N=3)	Asymmetry± SD (N=3)	%RSD (N=3)	Resolution ± SD (N=3)
Irbesartan	10.3 ± 0.12	9424±414	1.03±0.11	0.4	10.52 ± 0.02
Hydrochlorthiazide	3.5 ± 0.08	10291±512	1.09±0.10	0.3	

Table 3: RP-HPLC data of standard solution stability for Irbesartan and Hydrochlorthiazide

Standard solution stability				
Time (Hr)	Area		% Difference	
	Irbesartan	Hydrochlorthiazide	Irbesartan	Hydrochlorthiazide
0	205652	403806		
8	205053	404262	0.5	0.1
24	203433	403781	0.8	0.3
% Mean RSD			0.7	0.2

Table4: RP-HPLC data of sample solution stability for Irbesartan and Hydrochlorthiazide

Sample solution stability				
Time (Hr)	Area		% Difference	
	Irbesartan	Hydrochlorthiazide	Irbesartan	Hydrochlorthiazide
0	205041	405178		
8	203278	401523	0.5	0.6
24	201230	387126	0.9	1.0
% Mean RSD			0.8	0.8

Table 5: Peak purity data of RP-HPLC method for Irbesartan and Hydrochlorthiazide

Sample	3 point purity	
	Irbesartan	Hydrochlorthiazide
Standard Solution	0.9998	0.9999
Test solution	0.9997	0.9999

Table 6: RP-HPLC linearity data of Irbesartan and Hydrochlorthiazide.

Linearity range	Stock solution of linearity	Diluted to volume ml	Final conc Irbesartan (µg/ml)	Irbesartan Area ± SD
25%	2.50ml	100 ml	5	46519±95
50%	5.00ml	100 ml	10	98026±123
75%	7.50ml	100 ml	15	150543±181
100%	10.0 ml	100 ml	20	205653±216
125%	12.5 ml	100 ml	25	260541±345
150%	15.0 ml	100 ml	30	315623±424
Linearity range	Stock solution of linearity	Diluted to volume ml	Final conc Irbesartan (µg/ml)	Irbesartan Area ± SD
25%	2.50ml	100 ml	6.25	101236±101
50%	5.00ml	100 ml	12.50	202513±214
75%	7.50ml	100 ml	18.75	303845±321
100%	10.0 ml	100 ml	25.00	405236±429
125%	12.5 ml	100 ml	31.25	505236±524
150%	15.0 ml	100 ml	37.50	612361±608

Table 7: RP-HPLC repeatability and intermediate precision data of Irbesartan and Hydrochlorothiazide.

Repeatability									
Inj no	**Sample wt (mg)**	**Irbesartan (20 µg/ml)**				**Hydrochlorthiazide (25 µg/ml)**			
		Area	**% Assay**	**% Mean**	**% Mean**	**Area**	**% Assay**	**% Mean**	**% Mean**
1	1401.5	203413	99.4	99.6	0.4	408536	99.6	99.8	0.7
2	1402.5	202832	99.3			400753	99.4		
3	1408.5	205426	99.7			409019	99.4		
4	1406.5	205852	99.4			409421	98.2		
5	1380.5	203714	99.2			404951	99.5		
6	1392.6	208445	100.3			406214	99.5		
Intermediate precision									
1	1405.00	205516	99.6	99.5	0.8	401356	99.3	100.0	0.9
2	1406.50	205412	99.6			401495	99.4		
3	1407.00	206547	100.3			401486	99.5		
4	1402.50	206651	100.4			401492	99.6		
5	1403.50	202456	98.3			405062	100.5		
6	1403.20	203561	98.9			409178	101.5		

Table 8: RP-HPLC accuracy data of Irbesartan.

Level	**Placebo (mg)**	**Conc (µg/ml)**	**Amount of drug added (µg/ml)**	**Amount of drug recovered (µg/ml)**	**Area**	**Recovery (%) ± SD (N=3)**	**Mean (%)± SD**	**% RSD**
50%	1303.50	10	20.56	24.49	107562	100.1±0.2	99.2±0.6	0.6
	1306.56		20.66	24.39	107556	100.2±0.3		
	1304.98		20.76	24.80	107445	99.4±0.2		
100%	1302.28	20	40.02	39.94	205765	99.6±0.1	99.3±0.6	0.6
	1302.42		40.20	39.88	205471	99.7±0.2		
	1302.67		40.35	39.78	205562	98.7±0.1		
150%	1302.94	30	74.95	75.42	311835	100.0±0.2	100.4±0.5	0.6
	1302.86		75.15	75.47	312654	101.1±0.3		
	1302.52		75.25	75.42	313516	100.3±0.2		

Table 9: RP-HPLC accuracy data of Hydrochlorthiazide.

Level	**Placebo (mg)**	**Conc (µg/ml)**	**Amount of drug added (µg/ml)**	**Amount of drug recovered (µg/ml)**	**Area**	**Recovery (%) ± SD (N=3)**	**Mean (%) ± SD**	**% RSD**
50%	1302.50	12.5	24.85	24.89	201952	99.8±0.2	99.2±0.6	0.6
	1302.56		25.15	24.79	202163	99.0±0.1		
	1302.98		25.25	24.82	202442	98.7±0.2		
100%	1302.14	25	49.80	49.89	404162	99.5±0.3	99.5±0.5	0.5
	1302.50		50.10	49.90	402233	99.4±0.2		
	1302.81		50.30	49.91	404315	99.2±0.4		
150%	1302.94	37.5	74.85	75.22	612513	100.6±0.2	100.4±0.2	0.2
	1302.86		75.25	75.37	612956	100.4±0.3		
	1302.52		75.30	75.46	612512	100.2±0.3		

Table 10: Robustness data for Irbesartan and Hydrochlorthiazide.

Irbesartan robustness study. (%RSD For n=5 injections)								
Sys suit	**Temp -5^{0}C**	**Temp +5^{0}C**	Flow -10%	Flow - +10%	Org. - 2%	Org. +2%	pH = 3.2	pH = 2.8
0.1	0.1	0.1	**0.8**	0.1	**0.2**	**0.7**	**0.7**	**0.6**

Mean %RSD	0.3							
Hydrochlorthiazide robustness study. (%RSD For n=5 injections)								
Sys suit	**Temp -5^{0}C**	**Temp +5^{0}C**	Flow -10%	Flow - +10%	Org. - 2%	Org. +2%	pH = 3.2	pH = 2.8
0.1	0.9	0.5	**0.9**	0.7	**0.3**	**0.1**	**0.3**	**0.2**
Mean %RSD	0.4							

Table 11: LOD and LOQ RP-HPLC data for Irbesartan and Hydrochlorthiazide

Parameters	**Irbesartan**	**Hydrochlorthiazide**
LOD	0.2100 µg/ml	0.2000 µg/ml
LOQ	0.8150 µg/ml	0.6100 µg/ml

Table12: Summary of validation parameters of RP-HPLC method for simultaneous estimation of Irbesartan and Hydrochlorthiazide.

Parameters of validation	**Acceptance criteria**	**Irbesartsan**	**Hydrochlorthiazide**
Range of linearity	Follows Beer Lambert's law	5 – 30 µg/ml	6.25-37.5 µg/ ml
Correlation coefficient	r>0.999 or 0.995	0.9999	0.9999
Regression coefficient	r 2 > 0.999 or 0.995	0.9998	0.9999
LOD	S/N > 2 or 3	0.2100 µg/ml	0.2000 µg/ml
LOQ	S/N > 10	0.8250 µg/ml	0.6600 µg/ml
Repeatability	RSD < 2%	0.5%	0.8%
Intermediate precision	RSD < 2%	0.8%	0.9%
Accuracy	98- 102%	99.3% to 100.4%	99.2% to 100.4%
Specificity of blank, placebo	No interference	No interference of blank, placebo	No interference of blank, placebo
Solution stability	> 12 hour	Stable for 24 hr %RSD = 0.7%	Stable for 24 hr %RSD =0.9%
Robustness Flow rate (+ & -), Buffer pH (+ & -), Column temperature(+ & -), & Organic ratio (+ & -), in mobile phase	RSD NMT 2% in given condition	Complies %RSD 0.7% & 0.9% % RSD 0.3% & 0.2% % RSD 0.5% & 0.9 % % RSD 0.1% & 0.3%	Complies % RSD 0.1% & 0.8% % RSD 0.7% & 0.6% % RSD 0.1% & 0.1 % % RSD 0.7 % & 0.2%

Figure 1: RP-HPLC chromatogram of Irbesartan and Hydrochlorthiazide.

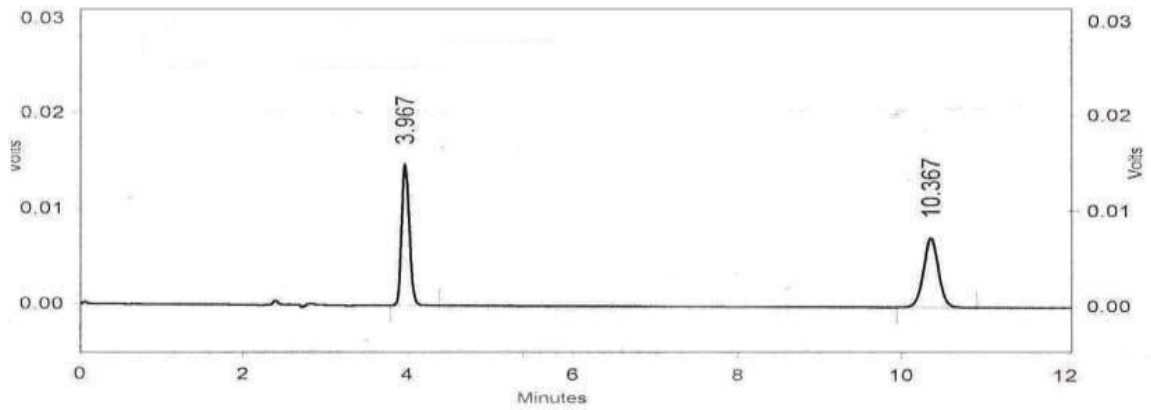


Figure 2: Calibration curve of Irbesartan by RP-HPLC analysis.

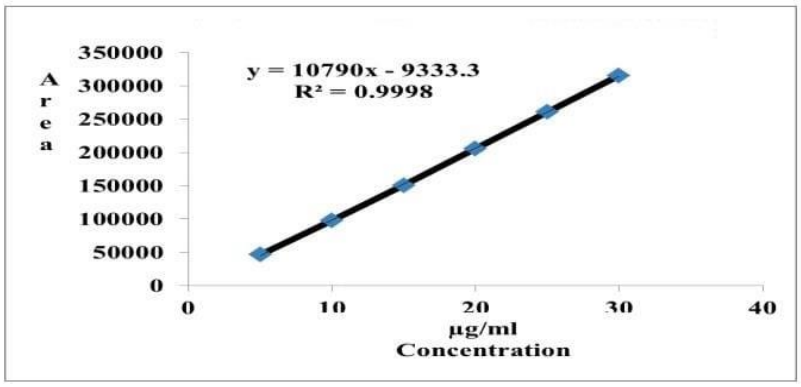
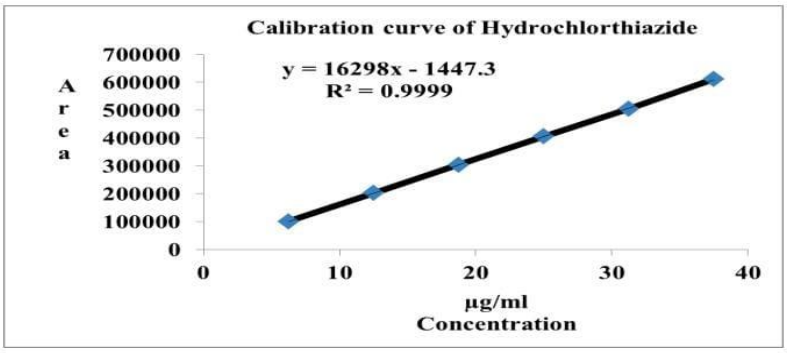


Figure 3: Calibration curve of Hydrochlorthiazide by RP-HPLC analysis.



RESULT AND DISCUSSION

The proposed method was found to be simple, accurate, precise and rapid for simultaneous estimation of Irbesartan and Hydrochlorothiazide in all dosage forms. The values of relative standard deviation are satisfactorily low and recovery was close to 100% which indicated accuracy and reproducibility of methods.

CONCLUSION

Validated RP HPLC method has been developed and successfully applied for simultaneous estimation of Irbesartan and Hydrochlorothiazide. Use of ACE C18 AR column which has high carbon loading and aromatic group bonded to C18 chain in column for proper peak separation for Irbesartan and Hydrochlorothiazide is key feature of developed method. Alkaline pH of buffer solution delayed elution of peaks from column compared to acidic pH of buffer solution. Hence this method can be used for analysis bulk and pharmaceutical dosage form.

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