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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF HYDROCHLOROTHIAZIDE AND IRBESARTAN IN COMBINED DOSAGE FORM BY RP HPLC

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ABSTRACT

A simple, precise, rapid and accurate RP-HPLC method has been developed for the estimation of Irbesartan and Hydrochlorothiazide in tablet dose forms having Labindia UV 3000 as detector by Isocratic technique, using Symmetry (C18) YOUNGLIN, 150mm×4.6mm column, Potassium dihydrogen orthophosphate as buffer and Buffer: Acetonitrile: Methanol (30:10:60) as mobile phase in various proportions in combination at 231nm and 273nm. The method was found to be linear (r>0.999), precise (RSD: 0.96 for Irbesartan, 0.905 for Hydrochlorothiazide) and accuracy (mean percentage recovery fields 99.13% for Irbesartan, 98.94% for Hydrochlorothiazide). The proposed HPLC method was simple, precise because of commonly used buffer and shorter run time (8 min). The mean percentage recovery (> 95%) indicates the reproducibility and accuracy of new developed method. The results obtained in this research work clearly indicated the method can easily and conveniently be adopted for the routine estimation and determination of combined dosage form of Irbesartan and Hydrochlorothiazide.

KEYWORDS: Irbesartan, Hydrochlorothiazide, Acetonitrile, Methanol, RP-HPLC, Mobile Phase.

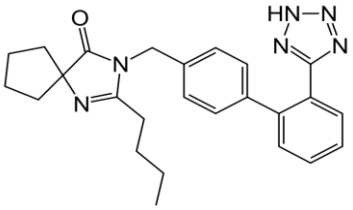
INTRODUCTION

Irbesartan is a non-peptide tetrazole[1] derivative and an angiotensin II antagonist that selectively blocks the binding of angiotensin II to the AT_1 receptor. It is used mainly for the treatment

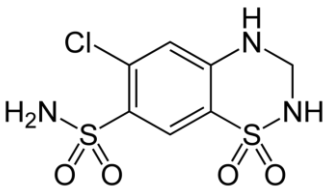
of hypertension. It works by relaxing blood vessels. It is also used in certain patients to treat kidney problems caused by diabetes (diabetic nephropathy). It is chemically 2-butyl-3-[p-(o-1H-tetrazol-5-ylphenyl) benzyl]-1, 3- Diazaspiro [4.4]non-1-en-4-one. Irbesartan is slightly soluble in alcohol and methylene and practically insoluble in water.

Hydrochlorothiazide belongs to the thiazide[2] class of diuretics, acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. The major site of action in the nephron appears on an electroneutral Na^+-Cl^- co-transporter by competing for the chloride site on the transporter. By impairing Na transport in the distal convoluted tubule, hydrochlorothiazide induces a natriuresis and concomitant water loss. This medication is used to treat high blood pressure. Lowering high blood pressure helps prevent strokes, heart attacks and kidney problems. It is chemically 6chloro-1,1-dioxo-6-3,4-dihydro-2*H*-1,2, 4-benzothiadiazine-7- sulfonamide. Soluble in water and methanol and insoluble in dilute mineral acids.

After through literature survey, the present method was developed. Many HPLC methods[3] have been reviewed using various Mobile phase in combinations with different elution times. The proposed method was done keeping in view economy and using cost effective mobile phase[4] and buffer solution and the retention time was also found to be less compared to the existing methods as per literature reviews.



Structure of Irbesartan



Structure of Hydrochlorothiazide

MATERIAL AND METHOD

Irbesartan (IRB) and Hydrochlorothiazide (HCT) were received gift samples from Lara Drugs Pvt Ltd, Hyderabad. Potassium dihydrogen phosphates, Methanol, orthophosphoric acid are from MERCK INDIA. All other solvents used were of analytical grade[5] and HPLC standard only.

Chromatographic Conditions

Mode of separation used in this procedure is Isocratic elution[6] technique. Mobile phase used is Phosphate buffer (p^H 6.25): Methanol: Acetonitrile (30:60:10). Column used is Symmetry C18 (4.6 x 150mm, 3.5µm) with flow rate of 0.7 ml / min and injection volume of 20 µl respectively.

Detection wave lengths are 231nm and 273nm with run time of 8 min.

Preparation of Buffer Solution[7]

Weighed 6.8 gm of KH_2PO_4 into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water. Adjuste the p^H to 6.25 with Sodium Hydroxide.

Mobile Phase

Mix a mixture of above buffer 300 ml (30%), 100 ml of Acetonitrile HPLC (10%) and 600ml of Methanol HPLC (60%) and degas[8] in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Standard Solution Preparation

Accurately weigh and transfer 10 mg of IRB working standard into a 10ml clean dry volumetric flask add about 10ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution A). Accurately weigh and transfer 10mg of HCT working standard into a 10ml clean dry volumetric flask add about 10ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution B). Further pipette 1.5 ml & 0.12ml of solution A & B respectively into a 10ml volumetric flask and dilute up to the mark with diluents.

Sample Solution Preparation

Accurately weigh and transfer 40.1 mg of IRB & HCT tablet powder into a 100ml clean dry volumetric flask add about 70ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette[9] 1ml of IRB and HCT of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Selection of wavelength

From the UV-visible spectrophotometric results, a detection wavelength of 273nm for Hydrochlorothiazide and 231nm for Irbesartan was selected.

ASSAY PROCEDURE

Standard preparations are made from the API and sample preparations are from formulation. Both sample and standard are injected for six homogenous samples. Drug in the formulation was estimated by taking standard as the reference. The average % Assay are calculated and found to be 99.71% and 99.67% for IRB and HCT respectively.

(**Figure 1), (Table 1), (Table 2)**

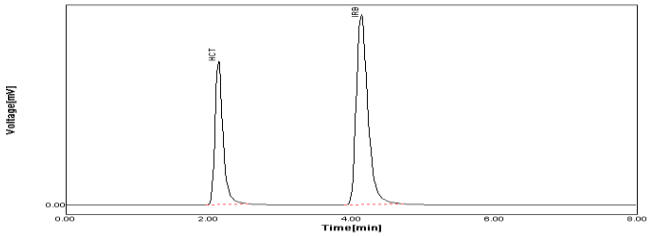


Figure 1: Chromatogram of Standard graph

Table 1: Percentage Label claim details for IRB and HCT

Drug	Label Claim	% Label Claim
IRB	150	99.71
HCT	12.5	99.67

Table 2: Summary results for Standard HCT & IRB

No	Name	RT[min]	Area[µV*s]	TP	TF
1	**HCT**	2.1000	541908.8	3187.79	1.2700
2	**IRB**	4.1667	1465536.5	4728.91	1.3200

RESULTS AND DISCUSSION

Experiment was conducted by taking various proportions of buffer solutions and graphs were plotted accordingly. Trail-1, buffer solution taken as Potassium dihydrogen orthophosphate into 1000ml of water (p^H adjusted to 3.5 ± 0.05 with Acetic acid) and mobile phase as Buffer: Acetonitrile: Methanol (20:50:30), not observed proper peak shape and consistent retention time. Trail-2, mobile phase combination was modified and taken as Buffer: Acetonitrile: Methanol (10:60:30), couldn't get the peak separation. Trail-3, buffer solution was changed to Potassium dihydrogen orthophosphate into 1000ml of water(p^H adjusted to 6.5± 0.05 with KOH) and mobile phase as Buffer:Acetonitrile:Methanol (50:10:40), faster elution of hydrochlorothiazide was observed. Trail-4, keeping the buffer solution same, mobile phase was changed to Buffer: Acetonitrile: Methanol (30:15:55), couldn't get consistent retention time. The above trials indicating that RT for the drug was not constant and elution time was faster which not prefered for the analysis. Finally, Trail-5, buffer solution was taken as Potassium dihydrogen orthophosphate into 1000ml of water (p^H adjusted to 6.25 with sodium hydroxide), mobile phase as Buffer: Acetonitrile: Methanol (30:10:60), the %RSD value, plate count and tailing factor results were found to be satisfactory. (**Table 3).**

Table 3: Summary results for Standard HCT & IRB

Injection	HCT		IRB	
S. No.	**RT**	**Area**	**RT**	**Area**
Inj-1	2.17	541666	4.15	1467239
Inj-2	2.13	546287	4.72	1455389
Inj-3	2.16	548734	4.16	1462836
Inj-4	2.13	542686	4.17	1465536
Inj-5	2.14	546739	4.16	1463968
Inj-6	2.15	549646	4.15	1472529
MEAN	2.146	545959.6	4.151	1464582.8
SD	0.016	3198.52	0.017	5640.24
% RSD	0.64	0.5858	0.36	0.38

METHOD VALIDATION[10], [11]

The method was validated in accordance with ICH[12] Guidelines.

System suitability[13]

It is defined to measure that can generate result of acceptable accuracy and precision. The system suitability was carried out after the method development and validation was completed. For these parameters like number of theoretical plates (N), tailing factor, RSD of peak area for repetitive injections was measured. (**Table 4).**

Table 4: System suitability parameters for Linearity curve

Parameters	IRB	HCT
Tailing factor (T)	1.27	1.32
Number of theoretical plate (N)	3187	4728
Retention time (R_t)	2.1	4.1
%RSD	0.64	0.36
Correlation coefficient	0.999	0.999

The tailing factors were found to be 1.27 and 1.32 respectively in limit of acceptance criteria.[14] The number of theoretical plates were found to be 3187, 4728 which were found to be in the acceptance criteria of not less than 2000. The %RSD values were found to be 0.64 and 0.36 which were found to be in the acceptance criteria limit of less than 2%. The correlation coefficient[15] was found to be 0.99 which is found to be in the acceptance criteria limit of less than 1%.

Accuracy[15]

Accuracy of the method was determined by recovery experiments. To the formulation, the reference standards of the drug were added at the levels of 80%, 100% and 120%. The recovery studies were carried out three times and the percentage recovery and percentage relative standard

deviation of the recovery for Irbesartan and Hydrochlorothiazide was calculated. The recovery results indicating that the test method has an acceptable level of accuracy for the assay of Irbesartan and hydrochlorothiazide was from 80% to 120% of test concentration. (**Figure 2), (Table 5).**

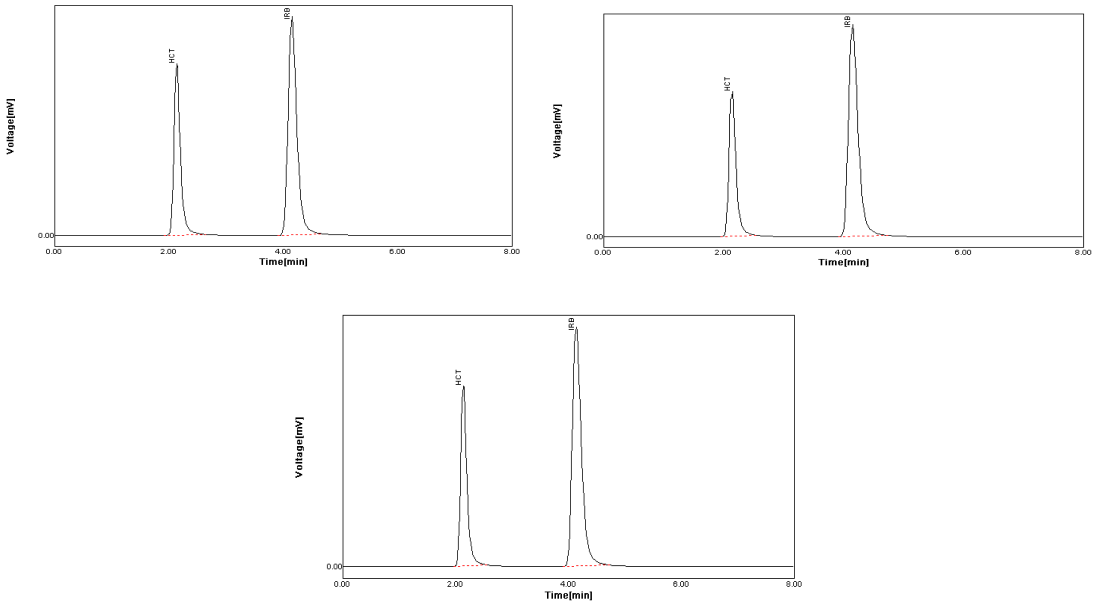


Figure 2: Chromatograms of Accuracy 80%, 100% and 120% solutions

Table 5: Summary results of Accuracy parameter for IRB and HCT

HCT							
Recovery Level	**Resultant Solution**	**Standard Injections**				**% Recovery**	**% RSD**
	(µg/ml)	**Inj-1**	**Inj-2**	**Inj-3**	**Average**		
80%	10.00	537039	538876	539193	538369.3	98.3059	0.216
100%	12.50	540574	547647	550652	546291	99.7524	0.947
120%	15.00	551039	549204	549039	549760.7	100.386	0.201
IRB							
80%	120.00	1437538	1443267	1433734	1438180	98.13336	0.333671
100%	150.00	1466358	1462835	1453672	1460955	99.68742	0.448243
120%	180.00	1484674	1493745	1480914	1486444	101.4267	0.443754

Precision[15]

Precision of the method was determined by injecting samples 6 times and checking the closeness of these values. Intermediate precision is the agreement of complete measurements when the same method is applied many times within the same laboratory. Intermediate precision was established for the same analytical samples of concentration 0.03 mg/ml. the analysis was done on different days. The results along with mean value for assay of IRB and HCT were shown. The %RSD value

from six preparations observed to be less than 2 and hence the result found to be satisfactory. (**Figure 3), (Table 6).**

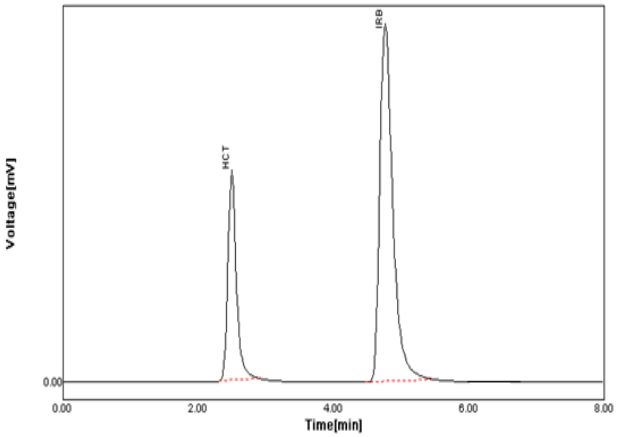


Figure 3: Chromatogram for Precision

Table 6: Summary results of Method Precision Parameter for HCT & IRB

Injection	HCT	IRB
I.P-1	545752	1472937
I.P-2	541983	1463381
I.P-3	546742	1454828
I.P-4	542984	1462937
I.P-5	547518	1463835
I.P-6	547307	1472935
MEAN	545381	1465142
SD	2347.69	6892.646
% RSD	0.43	0.470442

Linearity[16]

The linearity of calibration curves (peak area Vs concentration) in standard solution was checked over the concentration ranges. Injection volumes of 10 µg of each of standard solution were injected into HPLC system to get the chromatograms. The calibration line[16] was obtained by plotting peak area against concentration. The acceptance criteria was correlation coefficient should be not less than 0.99. The correlation coefficient values were found to be within the acceptance for both drugs.(**Figure 4), (Table 7).**

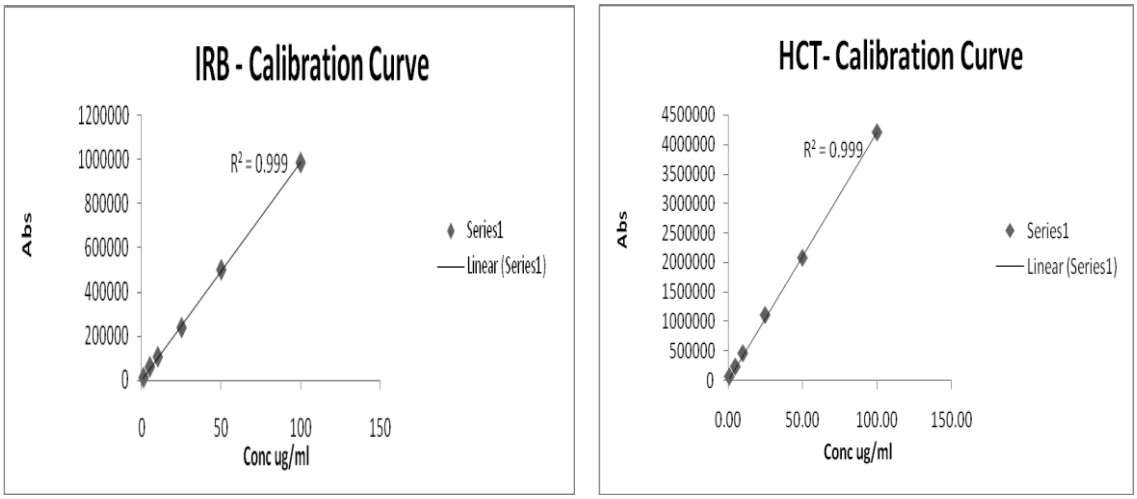


Figure 4: Calibration curves for IRB and HCT

Table 7: Summary results of Linearity parameter for HCT & IRB

Injection	HCT		IRB	
S. No.	Conc.	Area	Conc.	Area
1	1.00	58528	1.00	15892
2	5.00	226892	5.00	64394
3	10.00	453987	10.00	109209
4	25.00	1103945	25.00	240976
5	50.00	2076309	50.00	502091
6	100.00	4208734	100.00	985326

Limit of Detection and Limit of Quantification[17]

Both were performed for the procedure on sample containing very low concentration of analyte under the ICH guidelines. The % relative standard of assay preparation of Hydrochlorothiazide and Irbesartan should not be more than 2.0%. The % RSD value for LOQ was found to be less than 2 and hence the result found to be satisfactory. (**Figure 5), (Figure 6), (Table 8).**

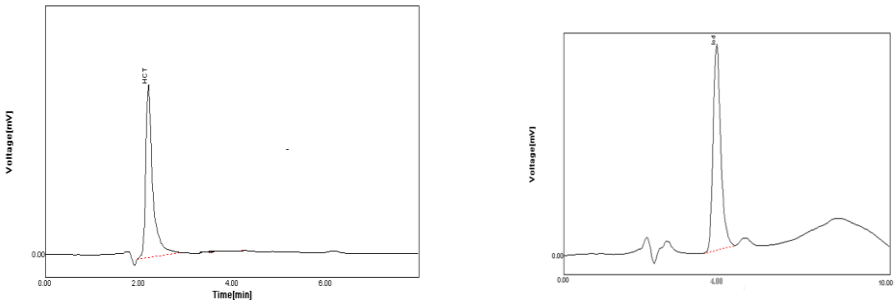


Figure 5: Chromatogram of LOD for HCT and IRB

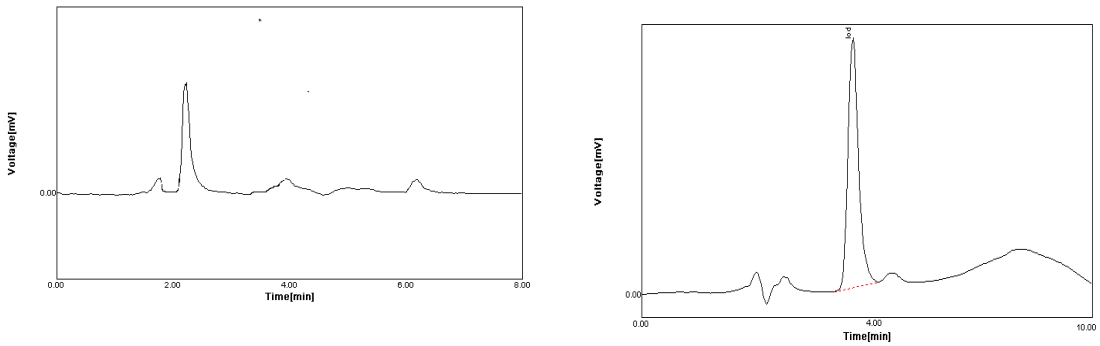


Figure 6: Chromatogram of LOQ for HCT and IRB

Table 8: Summary Results of LOQ parameter for HCT & IRB

Injection	HCT Area	IRB Area
Inj-1	3712	2362
Inj-2	3678	2383
Inj-3	3708	2398
Inj-4	3665	2356
Inj-5	3698	2405
Inj-6	3659	2356
MEAN	3686.667	2376.667
SD	22.51	21.75929
% RSD	0.61	0.915538
RT	2.1	4.1
Height	170 µV	160 µV

Robustness[18]

The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameters data after varying the HPLC pump flow rate (± 10%) and organic solvent content (± 5%), wavelength (± 2nm). None of the alterations caused significant changes in retention time, peak area, tailing factor and theoretical plates. (**Figure 7), (Table 9).**

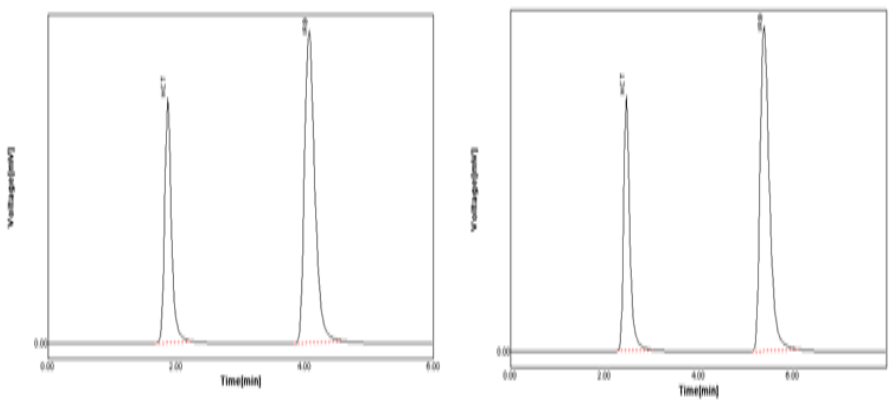


Figure 7: chromatogram for less flow and more flow

Table 9: Summary results of Robustness parameter for HCT & IRB

HCT					
PARAMETERS	**ADJUSTED TO**	**Area**	**RT**	**SD**	**% RSD**
FLOW RATE As per method 0.7ml/min	0.6 ml/min	640293	3.26	3714.75	0.58
	As it is	547831	2.13	1525.03	0.28
	0.8ml/min	441423	1.98	3378.03	0.77
Mobile phase composition (Buffer: Methanol: Acetonitrile 30:60:10)	Buffer: Methanol: Acetonitrile (25:62.5:12.5)	520049	2.96	4917.84	0.95
	As it is	548293	2.54	1643.98	0.30
	Buffer: Methanol: Acetonitrile (35:57.5:7.5)	583416	2.15	3774.98	0.65
IRB					
FLOW RATE As per method 0.7ml/min	0.6 ml/min	1677168	5.53	12528.55	0.75
	As it is	1464862	4.15	4761.47	0.33
	0.8ml/min	1269653	3.96	9759.52	0.77
Mobile phase composition (Buffer: Methanol: Acetonitrile 30:60:10)	Buffer: Methanol: Acetonitrile (25:62.5:12.5)	1345872	2.96	9087.52	0.68
	As it is	1465344	2.14	6947.01	0.47
	Buffer: Methanol: Acetonitrile (35:57.5:7.5)	1572340	2.15	9491.66	0.60

CONCLUSION

The proposed method was found to be simple, sensitive, rapid and economical for the determination of IRB and HCT in combined tablet formulation. The developed method was checked for the performance characteristics and has also been validated. Hence the method can easily and conveniently adopt for the estimation of combined dosage form of IRB and HCT.

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