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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF IRBESARTAN AND HYDROCHLOROTHIAZIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, accurate and precise RP-HPLC method was developed for the simultaneous estimation of Irbesartan (IRB) and Hydrochlorothiazide (HCTZ) in combination. A C 18 (Agilent ODS UG 5 Column 250mmX4.5 mm Dimensions) column with mobile phase composition Methanol: Acetonitrile: Buffer (10mM potassium dihydrogen phosphate pH6.8)(40:30:30%v/v/v) was used at isocratic mode and eluents were monitored at 264 nm. The retention times of IRB and HCTZ were 5.1 and 3.1min respectively. Irbesartan showed good linearity in the concentration range of 24-120 µg/ml with a correlation coefficient (R) of 0.9993 and 2-10 µg/ml for Hydrochlorothiazide with correlation coefficient (R) 0.9995 respectively. The proposed method was validated as per ICH guidelines and method showed good precision with percent relative standard deviation less than 2%. The assay values of Irbesartan and Hydrochlorothiazide were found to be 99.85 and 101% respectively and recovery values are within the limits of 98-102% indicating the proposed method was accurate and precise for the simultaneous estimation of Irbesartan and Hydrochlorthiazide in bulk and pharmaceutical dosage forms.

Keywords: Irbesartan, Hydrochlorothiazide, RP-HPLC, Simultaneous estimation.

INTRODUCTION

Irbesartan and hydrochlorothiazide combination is used in the treatment of hypertension. Irbesartan belongs to the category of Angiotensin -II receptor antagonist, and chemically it is 2-butyl -3({4-[2-(2H-1, 2, 3, 4-tetrazol-5yl) phenyl] phenyl} methyl) 1, 3-diazaspirol [4, 4] non-1-en-4-one and it is used in the treatment of hypertension and Diabetic nephropathy with an elevated serum creatinine and proteinuria in patients with type-2 diabetes. Chemically, Hydrochlorothiazide is 6-chloro- 3, 4-dihydro-7-sulfamoyl-2H-1, 2, 4-benzothiadiazine 1, 1-dioxide and it is used as diuretic[1-2]. The mechanism of action aims at inhibiting the absorption of sodium and chloride at the beginning of distal convoluted tubule. In this combination, hydrochlorothiazide is official in IP, BP and USP and a few analytical methods are reported for it. Irbesartan is official in USP. Literature survey reveals only few HPLC, spectrophotometric and HPTLC[3-8] methods were reported for the estimation of IRB either alone or in combination with other drugs in biological and formulation samples and few analytical methods were reported for the analysis of HCTZ by LC-MS. However, there was no validated RP-HPLC method published so far for the simultaneous estimation of IRB and HCTZ in bulk and dosage forms hence a suitable method was developed which will be used for routine analysis in quality control laboratories.

EXPERIMENTAL

Irbesartan (IRB) and Hydrochlorothiazide (HCTZ) were obtained as a gift samples. All the solvents which were used are of analytical grade.

Instrumentation: Agilent 1120 compact LC Chromatographic system with Variable Wavelength Programmable UV detector and Rheodyne Injector with 20µl fixed loop were used for the chromatographic separation. EZChrome software was used for data analysis. Chromatographic separation was carried out on a C_{18} column [Agilent ODS UG5 column 250mmX4.5mm]. For spectroscopic detection ELico double beam SL218 UV-VIS Spectrophotometer. AXIS AGN 204-PO electronic balance was used for weighing purpose. Ultrasonic bath sonicator, degasser is used

Chromatographic Conditions: Mobile phase consisting of Methanol: Acetonitrile: Buffer (10mM potassium dihydrogen phosphate pH-6.8) was used in isocratic mode and the mobile phase was filtered through nylon disc filter of 0.45µm (Millipore) and sonicated for 15 min before use. The flow rate was 1 ml/min and the injection volume was 20µL. VWD detection was performed at 264 nm and the separation was achieved at ambient temperature.

Preparation of Mobile Phase: Methanol: Acetonitrile: Buffer (10mM potassium dihydrogen phosphate pH-6.8) was mixed in the ratio of 40:30:30v/v/v.

Preparation of standard stock solution: Accurately weighed quantities of Irbesartan and Hydrochlorothiazide were dissolved separately in 10ml of methanol and make up to 10ml with methanol. Further dilution was made to obtain a combination of 120µg/ml of Irbesartan and 10µg/ml of Hydrochlorothiazide with mobile phase.

Preparation of sample stock solution: Ten tablets of IROVEL were accurately weighed and their average weight was determined. The tablets were crushed to fine powder and from the triturate, tablet powder equivalent to 10 mg of Irbesartan was weighed and transferred to 10 ml volumetric flask and dissolved in 5 ml Methanol and the content was kept in ultra-sonicator for 2min. Finally the volume was made up to the mark with methanol. The solution was filtered through Whatmann filter paper No.41 which gave a concentration of 1000 µg/ml and this solution was used sample stock solution.

VALIDATION OF THE METHOD [9]

Specificity: The method specificity was assessed by studying the chromatograms obtained from the sample solution. The method was found to be specific as none of the excipients interfered with the analytes of interest

System suitability: System suitability was carried out by injecting 120µg/ml of IRB and 10 µg/ml of HCTZ at different injection volumes in the range of 20-50µL with increment of injection volumes, the tailing factor and theoretical plate number was less than 1% and is satisfactory.

Linearity: The linearity responses are 24-120µg/ml and 2-10 µg/ml for Irbesartan and Hydrochlorothiazide respectively. The results were given in table1.

Precision: Precision was measured in terms of repeatability and it is carried out by injecting six replicates of 120µg/ml and 10µg/ml of Irbesartan and Hydrochlorthiazide respectively. The precision values were given in table 2

Accuracy: Accuracy of the method was ascertained by performing recovery studies. Recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different concentrations levels (50%, 100% and 150%) within the range of linearity. Results of recovery studies were given in table 3

LOD and LOQ: The LOD and LOQ values were determined by the formulae LOD = 3.3XS /m and LOQ = 10 S/m (Where, S is the standard deviation of the responses and m is mean of the slopes of the calibration curves)

Assay: An aliquot of 0.8ml from the sample stock solution was taken and made up to 10ml with water. The solution was injected three times in to the column. The amount present in each tablet was calculated by comparing the peakareas of standard with the test samples.

RESULTS AND DISCUSSION

The proposed method was found to be linear in the concentration range of 24-120µg/ml and 2-10µg/ml for Irbesartan and Hydrochlorothiazide respectively. The method was specific since excipients in the formulation did not interfere in the estimation of IRB and HCTZ. Accuracy of the method was indicated by the recovery values 98.6-100.6 for IRB and HCTZ. Precision is reflected by %RSD as 1.03 for IRB and 1.7 for HCTZ which are less than 2. The LOD and LOQ values were 0.13µg/ml and 0.41µg/ml

CONCLUSION

The proposed RP-HPLC method was validated as per International Conference on Harmonization (ICH) Guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of IRB and HCTZ using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The

method provides selective quantification of IRB and HCTZ without any interference. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

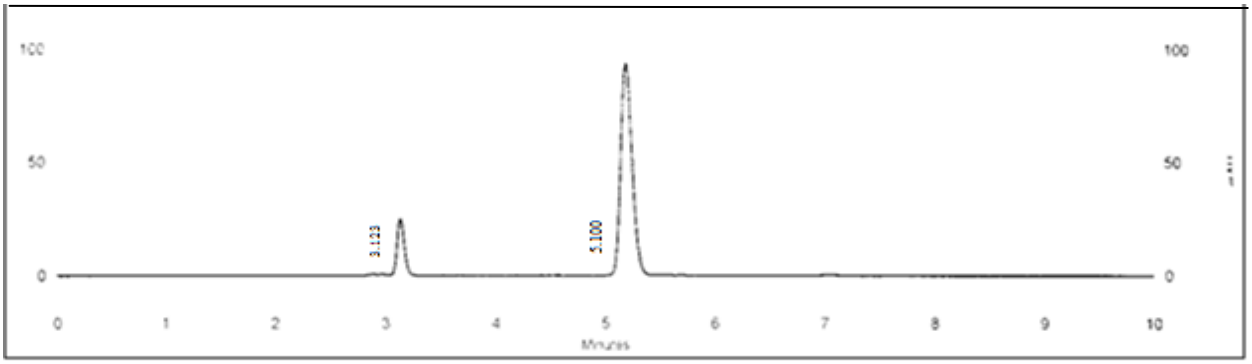

Fig 1: Typical chromatogram of Irbesartan and Hydrochlorothiazide

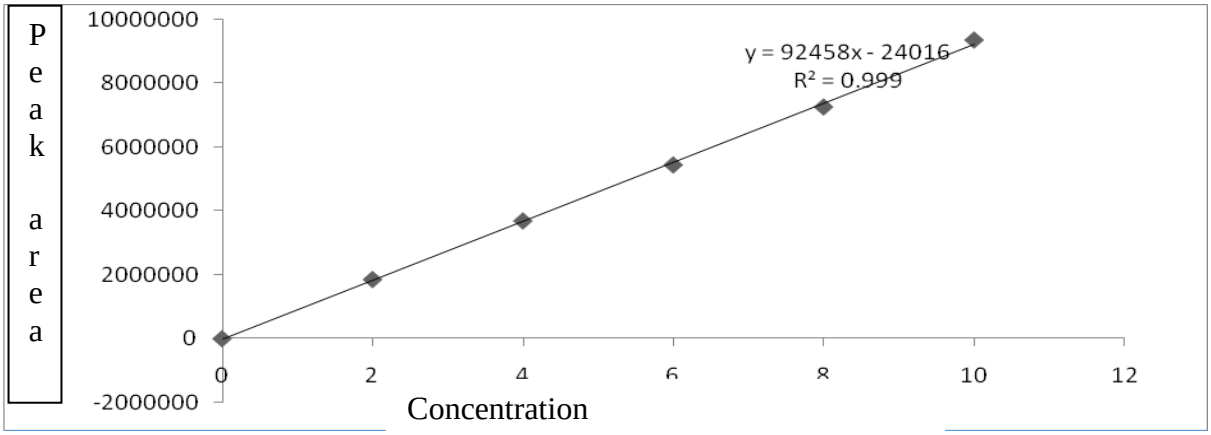

Fig 2: Linearity of Irbesartan

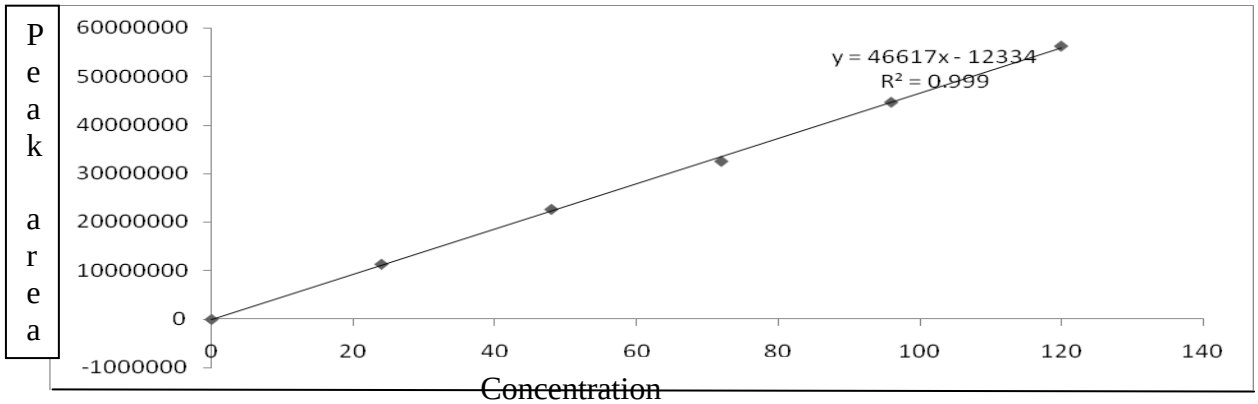

Fig 3: Linearity of Hydrochlorothiazide

Table-1 Linearity

S.no	Irbesartan			Hydrochlorothiazide		
	Conc. (µg/ml)	Retention time (min)	Peak area	Conc. (µg/ml)	Retention time (min)	Peak area
1	24	5.1	11390843	2	3.1	1854796
2	48	5.1	22692412	4	3.1	3988188
3	72	5.1	32589065	6	3.1	5140860
4	84	5.1	42760463	8	3.1	7257552
5	120	5.1	56316371	10	3.1	9451955
R^2	0.9993			0.9995		

Table2: Precision

Drug	Irbesartan	Hydrochlorothiazide
Mean	9432114	58250428
SD	97733.37	1029212
%RSD	1.03	1.7

Table-3 Accuracy

Drug	Level of recovery	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery
IRB	50%	36	35.5	98.66%
	100%	60	59.4	99%
	150%	84	84.58	100.6%
HCTZ	50%	3	3.01	100.5%
	100%	5	4.91	98.3%
	150%	7	6.99	99.8%

Table-4 System suitability studies

Parameters	Irbesartan	Hydrochlorothiazide	Limit
Retention time (min)	5.1	3.1	
Theoretical plates (N)	9751	6902	N > 2000
Tailing factor (T)	1.2	1.4	T of ≤ 2
Resolution (R_s)	5.0		R_s of > 2

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